Lipid oxidative changes in tradititional dry fermented sausage *Petrovská klobása* during storage

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Abstract

The influence of drying and ripeninig conditions (traditional and industrial) in the production of dry fermented sausage *Petrovská klobása*, on fatty-acid composition and oxidative changes in lipids, during 7 months of storage, was investigated. During the storage period, the sum of unsaturated fatty acids and the content of free fatty acids were significantly higher ($p < 0.05$), while the content of malondialdehyde was significantly lower in the sausage subjected to traditional conditions of drying and ripening. At the end of the storage period, contents of pentanal and hexanal in the sausage subjected to traditional conditions of drying and ripening (4.03 and 1.67 μg/g, respectively) were significantly lower ($p < 0.05$) in comparison with these contents in the sausage subjected to industrial conditions of drying and ripening. Traditional conditions of drying and ripening at lower temperatures have led to lower oxidative changes in lipids in traditional dry fermented sausage *Petrovská klobása* during storage period.

Keywords: *Petrovská klobása*, lipid oxidation, storage time.

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In many European countries, demand for traditional food is constantly increasing. Traditional fermented sausages are products mainly produced in small family communities across Europe [1]. *Petrovská klobása* is a traditional dry fermented sausage produced in Bački Petrovac (the province of Vojvodina, Serbia) exclusively from pork meat and fat with the addition of red hot paprika powder, salt, garlic, caraway and sugar. Red hot paprika has a dominant role in the formation of aroma of the *Petrovská klobása*. This product is also characterized by a dark-red colour and firm consistency [2]. Fermented sausages are products that contain a high percentage of fat. Fat is responsible for numerous properties of the fermented sausages. From a physiological aspect, fat is an important source of energy as well as of essential fatty acids and liposoluble vitamins [3]. Products formed during lipolysis and lipid oxidation have an important role in the formation of odour, taste and texture of the final product. However, fermented sausages also show some negative properties as a consequence of high content of animal fat [4]. Lipolysis is the first step in the process of autooxidation of free fatty acids [5]. Moreover, the oxidative degradation of lipids of meat and meat products involves the oxidation of unsaturated fatty acids, especially polyunsaturated fatty acids and cholesterol [6]. Polyunsaturated fatty acids having three or more double bonds are primarily tied to phospholipids and are important for the development of the characteristic flavor state of food. The free radicals formed in lipid oxidation ($R^{\bullet}$) react with oxygen producing peroxy radicals ($ROO^{\bullet}$). In this initial process $ROO^{\bullet}$ react with several RH resulting in lipid hydroperoxides (ROOH), which are the main primary products of oxidation [7––9]. Moreover, during secondary oxidation changes in free fatty acids, compounds such as aldehydes, ketones, carboxylic acids are being created. Aldehydes are the main products formed during the lipid oxidation. In addition to the important role in the formation of aroma, aldehydes have also toxic properties. Even in small amounts aldehydes disturb the favorable sensory properties of food [10, 11, 12]. These compounds are very reactive in redox transformation and are intermediates in many biochemical reactions. Also, many aldehydes formed during the smoking process or from lipid peroxides are carcinogenic and can cause diseases of the digestive tract [13]. Propanal and hexanal are the most commonly used indicators of lipid oxidation in food due to their higher oxidative stability in detection compared to unsaturated aldehydes [14,15]. Propanal is a typical product of the n-3 oxidation and hexanal is a product of oxidative degradation of n-6 polyunsaturated fatty acids [16], while octanal is most probably a product of secondary oxidation of oleic acid [13]. Ansorena *et al*. [11], Misharina *et al*. [17] and Valencia *et al*. [9] studied the changes in the content of alde-

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hydes as indicators of lipid oxidation in the fermented sausages during the storage period.

Vaštag *et al.* [18], Tasić *et al*. [2] and Ikonić *et al.* [19] studied the changes in proteins at different stages of ripening to the final degradation products (biogenic amines) during drying and ripening of *Petrovská klobása*, while Danilović *et al*. [20] dealt with identification of functional microflora in traditional dry fermented sausage *Petrovská klobása*.

However, it is little known about lipid oxidative changes in traditional dry fermented sausage *Petrovská klobása* during processes of drying, ripening and storage. The aim of this study was to comparatively examine the effect of drying and ripening methods (in the traditional and industrial conditions) and storage time on fatty-acid composition and oxidative changes in lipids of the traditional dry fermented sausage *Petrovská klobása*.

EXPERIMENTAL

Material

Sausages were produced in the winter period by using traditional manufacturing technology. Stuffing for the experimental sausages was made from chilled lean pork and fat in relation 85:15. Pork and firm fat tissue were grounded to pieces the size of 10 mm, and then the following ingredients were added: 2.50% red hot paprika powder, 1.80% salt, 0.20% raw garlic paste, 0.20% caraway and 0.15% crystal sugar. Starter cultures were not added so the sausages were subjected to spontaneous fermentation. Stuffing was hand-mixed, using a specific technique of tipping over and squashing for 10 min. The made stuffing was then filled into collagen casings (diameter of 55 mm). The sausages were subjected to straining during 24h. Subsequently sausages were smoked in chamber in the traditional way for 12 days with breaks. The atmospheric conditions during smoking were: 5–10 °C and *RH* 75–85%. After the smoking process, sausages were divided into 2 groups (T and I). Sausages of the T group were subjected to uncontrolled drying and ripening in traditional conditions *(t* = 0–10 °C, *RH* 95–80%) until achieving the moisture content of 35% (90 days). Sausages of I group were subjected to controlled drying conditions *(*8–10 °C, *RH* 90–75%) until achieving the moisture content of 35% (60 days). After the drying process, both groups of sausages were stored, *t* = 10 °C, *RH* = 75%, for 7 months.

Methods

Fatty acid profile determination

The method of Folch *et al.* [21] was used for the extraction of lipids from sausages. The fatty acid composition was determined by gas chromatography. For the preparation of fatty acid methyl esters, KOH/ /methanol was used. A Perkin–Elmer Varian, series 1400 gas chromatograph fitted with a packed column (3 m×3.0 mm, a stationary phase GP 10% SPTM-2330 on inert carrier 100/120 Chromosorb WAW) and flame ionization detection was used (Perkin-Elmer, Waltham, Massachusetts, USA). The temperature of both the injection port and the detector was 250°C. The carrier gas was N_2, with flow rate of 20 mL/min. The sample volume was 2.0 μL. The identification of the fatty acid methyl esters was by comparison of the retention times of peaks in the sample with those of standard pure compounds (Sigma-Aldrich Chemical, St. Louis, MO, USA). Fatty acids methyl esters were quantified as percentage of total methyl esters.

Determination of total fat

Total fat content of sausages was determined by application of ISO standard method [22].

Determination of free fatty acids

Free fatty acids content determined using the ISO standard method [23] and calculated as mg KOH/g lipid.

TBARS determination

TBARS (2-thiobarbituric acid reactive substances) test was performed using to the method of Bostoglou *et al*. [24], with modifications. Total volume of TCA was added to the sample and extraction was performed in ultrasonic bath XUB 12 (Grant Instruments, Cambridge, UK) [8]. Spectrophotometer Jenway 6300 (Jenway, Felsted, United Kingdom) was used. TBARS values were expressed as milligrams of malondialdehyde per kilogram of sample.

Aldehydes determination

Static headspace gas chromatographic (SHS–GC) analyses were performed on Agilent 7890A GC System (Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary split/split less inlet, total electronic pneumatic control of gas flow, headspace auto sampler and FID. Static headspace (SHS) sampling was performed with the headspace sampler, CombiPAL System (CTC Analytics, Zwingen, Switzerland). A 2.5 mL HS syringe for CombiPAL was used, for the injection of 2.0 mL of vapor phase from the 10 mL headspace vials. Chromatographic conditions and aldehydes standard preparation is performed according to Mandić *et al*. [25]. Homogenized sample was accurately weighed (2.00 g) into 10 mL scruecapped headspace vial.

Statistical analysis

Statistical analysis was carried out using STATISTICA 8.0 (StatSoft, Inc., Tulsa, OK, USA). All data were presented as mean value with their standard deviation indicated (mean ± *SD*). Variance analysis (ANOVA) was

performed, with a confidence interval of 95% ($p < 0.05$). Means were compared by t-test and Duncan's multiple range test.

RESULTS AND DISCUSSION

Fatty-acid composition of sausages subjected to traditional (sausage T) and industrial conditions (sausage I) of drying and ripening at the end of the drying process as well as after 2 and 7 months of storage are shown in Table 1.

At the end of the drying process sums of saturated fatty acids in sausages T and I groups amounted to 33.28 and 33.74%, respectively. These values were not significantly different ($p > 0.05$). However, during the entire period of storage the amount of saturated fatty acids was significantly lower ($p < 0.05$) in sausages produced in traditional conditions (sausage T), compared to those in sausages produced in industrial conditions (sausage I) of drying and ripening. Content of oleic acid (C18:1) in sausages of T and I groups was 45.69 and 45.22%, respectively. These values did not change significantly ($p > 0.05$) during storage period. Similar results were also achieved by Ansorena *et al*. [11]. There was no significant difference ($p > 0.05$) in the content of oleic acid between the investigated sausages neither at the end of the drying process nor during storage period. Linoleic and linolenic acids contents did not differ significantly ($p > 0.05$) during storage period in sausages of T and I groups. Obtained results are likely caused by good oxidative stability of sausages during the storage period [9]. Except for differences in the content of linolenic acid after 2 months of storage, there were no significant differences ($p > 0.05$) in the content of polyunsaturated fatty acids (C18: 2 and C18: 3), nor in the sum of polyunsaturated fatty acids

Table 1. The fatty acid composition in traditional dry fermented sausage Petrovská klobása *during storage; xy – the values of the same column significantly differ with 95% probability (p < 0.05); abc – the values of the same row significantly differ with 95% probability (p < 0.05)*

Fatty acid	Sausage	End of drying	2 months	7 months
C14:0	T	1.14±0.00yb	1.19±0.00ya	0.96±0.03yc
	I	1.20±0.01x	1.25±0.04x	1.35±0.24x
C16:0	T	20.38±0.33	20.58± 0.32	19.98± 0.37y
	I	20.15±0.39b	21.14±0.29a	20.73±0.17xab
C17:0	T	0.15±0.01yc	0.34±0.02yb	0.45±0.00xa
	I	0.36±0.03xb	0.52±0.01xa	0.18±0.01yc
C18:0	T	11.61± 0.09yb	12.51± 0.09a	11.65±0.06b
	I	12.02±0.08xb	12.43±0.08a	11.78±0.20b
C16:1	T	2.48±0.06	2.52±0.11x	2.51±0.03x
	I	2.30±0.45a	1.97±0.02yb	1.83±0.02yb
C17:1	T	0.23±0.02yb	0.34±0.02xa	0.34±0.05xa
	I	0.35±0.06xa	0.24±0.01yb	0.24±0.02yb
C18:1	T	45.69±0.32	44.96±0.31	45.33±0.31
	I	45.22±0.22	44.94±0.23	45.18±0.34
C20:1	T	1.31±0.02yb	1.25±0.04yb	1.43±0.04ya
	I	1.38±0.04xb	1.38±0.03xb	1.63±0.27xa
C18:2	T	15.98±0.16a	15.22±0.14b	16.28±0.34a
	I	15.99±0.14a	14.95±0.33b	16.01±0.11a
C18:3	T	1.00±0.02	1.05±0.01x	1.08±0.11
	I	0.97±0.04	0.86±0.01y	1.05±0.27
ΣSFA	T	33.28±0.42b	34.62±0.22ya	33.01±0.34yb
	I	33.74±0.35c	35.35±0.32xa	34.05±0.27xb
ΣUFA	T	66 .69±0.33a	65.33±0.11xb	66.96±0.43xa
	I	66.20±0.03a	64.34±0.12yc	65.93±0.14yb
ΣPUFA	T	16.98± 0.15b	16.26±0.14c	17.36±0.23a
	I	16.96±0.11a	15.81±0.32b	17.06±0.16a
ΣUFA/ΣSFA	T	2.00±0.03a	1.89±0.01xb	2.03±0.01xa
	I	1.96±0.02a	1.82±0.02yb	1.94±0.01ya
ΣPUFA/ΣSFA	T	0.51±0.01b	0.47±0.00xc	0.53±0.00xa
	I	0.50±0.00a	0.45±0.01yb	0.50±0.01ya

between T and I sausage groups. Moreover, after 2 and 7 months of storage, the sum of unsaturated fatty acids, relation U/S and P/S, were significantly higher (p < 0.05) in sausages produced in traditional conditions of drying and ripening. Thus, lower temperatures during the drying process have led to lower oxidation changes in unsaturated fatty acids in the sausage produced in traditional conditions of drying and ripening. On the other hand Summo *et al*. [26] and Rubio *et al*. [27] found that during prolonged storage there is no significant change in fatty acid composition in fermented sausages.

Total fat content at the end of the drying process, and then until the end of storage period ranged in an interval of 31.23–41.08% in sausage T and from 32.67 to 44.93% in sausage I (Table 2).

Obtained values for total fat content are in accordance with data from literature for similar products in the type of fermented sausages [28]. During storage period, the total fat content was significantly lower (p < 0.05) in sausage produced in traditional conditions of drying and ripening (sausage T) in relation to this content in the sausage produced in industrial conditions of drying and ripening (sausage I). Obtained distinctions are likely a consequence of fast drying of the sausages subjected to industrial conditions of drying and ripening. Free fatty acid content at the end of the drying process were within the range of 7.11 mg KOH/g of lipids for the sausage I and 14.62 mg KOH/g of lipids for the sausage T (Table 2). The obtained results are in agreement with the results of Vukovic *et al*. [28] and Muguerza *et al.* [29], for similar products in the type of fermented sausages. In both sausages during the entire storage period, free fatty acid content was significantly increasing (p < 0.05). The increase in free fatty acids content is probably the result of endogenous enzymes activity as well as the activity of enzymes of microorganisms [5]. Moreover, lipolitic changes are followed by oxidative changes that compounds such as unsaturated fatty acids and cholesterol are easily subjected to [29]. Malondialdehyde is a typical degradation product formed during lipid oxidation of polyunsaturated fatty acids [30]. At the end of the drying process in sausages produced in traditional and industrial conditions of drying and ripening, the content of malondialdehyde was 0.79 and 1.25 μg/g, respectively (Table 3).

These results are in agreement with literature data [9,31] but in contrast with the results of other authors [27,32]. Unlike the sausage I, in the sausage T, malondialdehyde content was reduced after 2 months of storage period. Malondialdehyde values after 7 months of storage period were 0.16 μg/g for sausage T and 0.93 μg/g for sausage I, and were significantly lower (p < < 0.05) compared to the determined value of malon-

Table 2. Total fat content and free fatty acid content in traditional dry fermented sausage Petrovská klobása *during storage; xy – the values of the same column significantly differ with 95% probability (p < 0.05); abc – the values of the same row significantly differ with 95% probability (p < 0.05)*

Parameter	Sausage	End of drying	2 months	7 months
Total fat, %	T	31.23±0.46yc	35.24±0.25yb	41.08±0.33ya
	I	32.67± 0.29xc	39.66±0.23xb	44.93±0.27xa
Free fatty acid content, mg KOH/g lipid	T	14.62±0.01xc	26.02±0.01xb	35.09±0.01xa
	I	7.11±0.01yc	13.93±0.01yb	28.15±0.01ya

Table 3. Lipid oxidation parameters in traditional dry fermented sausage Petrovská klobása *during storage; xy – the values of the same column significantly differ with 95% probability (p < 0.05); abc – the values of the same row significantly differ with 95% probability (p < 0.05)*

Lipid oxidation parameter	Sausage	End of drying	2 months	7 months
TBARS, mg malondialdyde/kg	T	0.79±0.02ya	0.36±0.01yb	0.16±0.02yc
	I	1.25±0.00xb	1.63±0.02xa	0.93±0.00xc
Aldehydes content, μg/g				
Propanal	T	1.86±0.53b	0.94±0.01yb	32.59±1.57a
	I	2.68±0.26b	4.80±0.10xb	44.32±10.67a
Pentanal	T	1.16±0.38c	2.75±0.12xb	4.03±0.25ya
	I	1.61±0.08b	0.89±0.06yb	9.52±2.40xa
Hexanal	T	0.12±0.08b	0.05±0.00yb	1.67±0.07ya
	I	0.05±0.00b	0.22±0.01xb	4.94±1.29xa
Heptanal	T	0.24±0.07c	1.65±0.09xa	0.52±0.02b
	I	0.30±0.12c	1.40±0.05ya	0.86±0.21b
Octanal	T	3.24±1.26xa	0.22±0.03yb	0.35±0.01b
	I	0.87±0.11ya	0.50±0.03xb	0.61±0.17b

dialdehyde in sausages of T and I groups after 2 months of storage. Reduction of malondialdehyde values during storage was probably the result of interaction of malondialdehyde with compounds such as sugars, nitrites, amino acids [33]. Furthermore, malondialdehyde values at the end of the drying process as well as during the entire period of storage in the tested sausages were significantly lower ($p < 0.05$) in sausage subjected to traditional conditions of drying and ripening. The content of malondialdehyde is negatively correlated with the free fatty acid content in both sausages at the end of the drying process as well as during the entire period of storage. Berger *et al*. [34] suggest that lower values of free fatty acids content may be a result of intense oxidative changes. Obtained results show that oxidation of free fatty acids in the traditional dry fermented sausage *Petrovská klobása* during prolonged storage period (7 months) is slower when sausages are processed under conditions of drying and ripening at lower temperatures.

Table 3 shows aldehyde content at the end of the drying process and during 2 and 7 months of storage. Aldehydes are bearers of a wide range of fragrances and flavors in food [10]. Propanal was the most dominant aldehyde at the end of the drying process as well as during storage period in both groups of sausages. It is in agreement with literature data [15]. At the end of the drying process, the content of octanal was significantly higher ($p < 0.05$) in the sausage produced in traditional conditions of drying and ripening, while the content of propanal, pentanal, hexanal and heptanal in this sausage was not significantly different ($p > 0.05$) in relation to the sausage produced in industrial conditions of drying and ripening. After 2 months of storage, the content of propanal ranged in an interval of 0.94 μg/g in the sausage T to 4.80 μg/g in the sausage I. After that, the content of propanal was significantly increased ($p < 0.05$) in both groups of sausages. The content of hexanal in the tested sausages of T and I groups, after 2 months of storage was 0.05 and 0.22 μg/g, respectively. The obtained results of hexanal content are very similar to values that were found in fermented sausages by Josquin *et al*. [15] and Misharina *et al*. [17]. Following two months of storage, content of propanal, hexanal and octanal was significantly lower ($p < 0.05$) in the sausage produced in traditional conditions of drying and ripening, while the content and pentanal and octanal was significantly higher ($p < 0.05$) in this sausage in relation to the content of aldehydes in the sausage produced in industrial conditions of drying and ripening (Table 3). Moreover, after 7 months of storage, the content of propanal, pentanal and hexanal in both groups of sauseges was significantly increased ($p < 0.05$). Significant increase in the content of aldehydes during storage period in fermented sausages was determined by Valencia *et al*. [9], Ansorena *et al*. [11] and Misharina *et al*. [17]. However, the content of heptanal was significantly ($p < 0.05$) decreased after 2 months of storage while octanal showed a tendency to decrease at the completion of the drying process. Heptanal and octanal decreasing trend was present in both sausages, which is probably a consequence of an interaction of aldehydes with amino and -SH groups of proteins. These reactions lead to lower evaporability of aldehydes in detection [35]. Additionally, longer storage period, especially at higher temperatures, can lead to degradation of malondialdehyde and other aldehydes, leading to the formation of volatile compounds of lower molecular weight [36]. After 7 months of storage, propanal content ranged in an interval of 32.59 μg/g in the traditional, to 44.32 μg/g in the sausage produced under industrial conditions of drying and ripening. There was no significant difference ($p > 0.05$) between the obtained values of propanal content. Obtained results of propanal content in *Petrovská klobása* were much higher than the values found in fermented sausages by Josquin *et al*. [15]. On the other hand, the content of pentanal and hexanal was significantly lower ($p < 0.05$) in the traditional than in sausage produced in industrial drying conditions. Moreover, after 7 months of storage, values of heptanal in sausages of T and I groups amounted to 0.52 and 0.86 μg/g, respectively. The obtained values were similar to values determined in sausages from the Mediterranean by Ansorena *et al*. [11] and Demeyer *et al*. [37]. After 7 months of storage numerical values of the contents of heptanal and octanal in sausages produced in the traditional drying and ripening conditions were less than those values in sausages produced under industrial conditions of drying and ripening. However, the obtained values were not significantly different ($p > 0.05$). Lower values of the content of aldehyde after 7 months of storage in sausage T indicate better oxidative stability of the sausage produced in conditions of slower drying and ripening at lower temperatures.

CONCLUSION

During storage period, the sum of unsaturated fatty acids and the content of free fatty acids in the sausage produced in traditional conditions were significantly higher ($p < 0.05$) compared to the sausage produced in industrial conditions of drying and ripening while the sums of polyunsaturated fatty acids between the investigated sausages were not significantly different ($p > 0.05$). At the end of the storage period content of malondialdehyde and saturated aliphatic aldehydes was lower in the sausage produced in the traditional conditions of drying and ripening.

Obtained results indicate that conditions of slower drying and ripening at lower temperatures result in less lipid oxidative changes in traditional dry fermented sausage *Petrovská klobása* during prolonged storage period (7 months).

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IZVOD

OKSIDATIVNE PROMENE NA LIPIDIMA TRADICIONALNE SUVE FERMENTISANE KOBASICE *PETROVSKÁ KLOBÁSA* TOKOM SKLADIŠTENJA

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U radu je ispitan uticaj načina sušenje i zrenja (u tradicionalnim i industrijskim uslovima) tradicionalno proizvedene fermentisane kobasice (*Petrovská klobása*) tokom 7 meseci skladištenja na masno-kiselinski sastav i oksidativne promene na lipidima. Oksidativne promene na lipidima utvrđene su preko vrednosti malondialdehida i sadržaja zasićenih alifatičnih aldehida. Tokom skladištenja suma nezasićenih masnih kiselina i sadržaj slobodnih masnih kiselina izražen preko vrednosti kiselinskog broja bili su statistički značajno veći ($p < 0,05$) u kobasici podvrgnutoj tradicionalnim uslovima sušenja i zrenja. Sadržaj malondialdehida u kobasici proizvedenoj u tradicionalnim uslovima kretao se u intervalu od 1,27 mg/kg na kraju procesa sušenja do 0,16 mg/kg na kraju vremena skladištenja i bio je statistički značajno manji ($p < 0.05$) u odnosu na taj sadržaj u kobasici podvrgnutoj industrijskim uslovima sušenja i zrenja. Na kraju vremena skladištenja sadržaj pentanala i heksanala u kobasici podvrgnutoj tradicionalnim uslovima (4,03 i 1,67 μg/g, redom) bio je statistički značajno manji ($p < 0,05$) u poređenju sa tim sadržajem u kobasici podvrgnutoj industrijskim uslovima sušenja i zrenja (9,52 i 4,94 μg/g, redom). Tradicionalni uslovi sušenja i zrenja pri nižim temperaturama doveli su do manjih oksidativnih promena na lipidima Petrovačke kobasice.

Ključne reči: *Petrovská klobása* • Oksidacija lipida • Vreme skladištenja